

Amendments to the Specification

Please replace the paragraph on page 4, lines 22-23, with the following paragraph:

Figure 1. Presentation of the nucleic acid sequence (SEQ ID NO: 1) and the amino acid sequence (SEQ ID NO:2) of the mutant PTH1R receptor, rδNt.

Please replace the paragraph spanning page 6, line 23, and page 7, line 3, with the following paragraph:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the rδNt receptor polypeptide, a novel, mutant PTH1R receptor polypeptide, having the amino acid sequence shown in Figure 1 (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The rδNt protein of the present invention shares sequence homology with previously identified non-mutant PTH1R and PTH2R sequences. The nucleotide sequence shown in Figure 1 (SEQ ID NO:1) was obtained by sequencing a cDNA clone (rδNt), which was deposited on [[____]] December 28, 1999, at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number [[____]] PTA-1136.

Please replace the paragraph on page 8, lines 6-29, with the following paragraph:

As indicated, the present invention also provides the mature form(s) of the rδNt receptor of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However in some cases, cleavage of a secreted protein is not entirely

uniform, which results in two or more mature species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature rδNt polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. [[____]] PTA-1136 and as shown in Figure 1 (SEQ ID NO:2). By the mature rδNt protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. [[____]] PTA-1136 is meant the mature form(s) of the rδNt receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature rδNt receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. [[____]] PTA-1136 may or may not differ from the predicted "mature" rδNt protein shown in Figure 1 (amino acids from about 23 to about 435) depending on the accuracy of the predicted cleavage.

Please replace the paragraph spanning page 10, line 24, and page 11, line 7, with the following paragraph:

In another aspect, the invention provides isolated nucleic acid molecules encoding the rδNt polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. [[____]] PTA-1136 on [[____]] December 28, 1999. Preferably, the nucleic acid molecule will encode the mature polypeptide encoded by the above-described deposited cDNA clone. In a further embodiment, a nucleic acid molecule is provided encoding the rδNt polypeptide or the rδNt polypeptide lacking the N-

terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or the nucleotide sequence of the rδNt cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the rδNt gene in human tissue, for instance, by Northern blot analysis.

Please replace the paragraph on page 12, lines 16-24, with the following paragraph:

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit Nos. _____ or _____ PTA-1136, PTA-1138, PTA-1139, PTA-1140, PTA-1142, PTA-1137 or PTA-1141. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1xSSC at about 65°C.

Please replace the paragraph spanning page 14, line 21, and page 15, line 9, with the following paragraph:

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length rδNt polypeptide having

the complete amino acid sequence in SEQ ID NO:2, including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature rδNt receptor (full length polypeptide with the leader removed) having the amino acid sequence at positions from about 23 to about 435 in SEQ ID NO:2; (d) a nucleotide sequence encoding the full-length rδNt polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. [[____]] PTA-1136; (e) a nucleotide sequence encoding the mature rδNt receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883; (f) a nucleotide sequence encoding the rδNt receptor extracellular domain; (g) a nucleotide sequence encoding the rδNt receptor transmembrane domain; (h) a nucleotide sequence encoding the rδNt receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h).

Please replace the paragraph spanning page 25, line 7, and page 26, line 10, with the following paragraph:

Intracellular cAMP accumulation is measured as described previously (Abou-Samra *et al.*, *J. Biol. Chem.* 262:1129, 1986). Cells expressing the rδNt receptor grown in 24-well plates are rinsed with culture medium containing 0.1% BSA and 2 mM IBMX. The cells are then incubated with a test compound for 60 min. at 21°C. The supernatant is removed and the cells immediately frozen by placing the whole plate in dry ice powder. Intracellular cAMP is extracted by thawing the cells in 1 ml of 50 mM HCl and analyzed by a specific radioimmunoassay using an anti-cAMP antibody (e.g., Sigma, St. Louis, Mo). A cAMP

analog (2'-O-monosuccinyl-adenosine 3':5'-cyclic monophosphate tyrosyl methyl ester, obtained from Sigma) which is used a tracer for cAMP is iodinated by the chloramine T method. Free iodine is removed by adsorbing the iodinated cAMP analog onto a SEP-PAK C18 ~~Sep-pak~~ cartridge (Walters, Milford, Mass.). After washing with dH₂O, the iodinated cAMP analog is eluted from the SEP-PAK ~~Sep-pak~~ cartridge with 40% acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA), and injected into a C18 reverse phase HPLC column (Waters). The column is equilibrated with 10% ACN in 0.1% TFA, and eluted with gradient of 10-30% ACN in 0.1% TFA. This allows separation of the mono-iodinated cAMP analog from the non-iodinated cAMP analog. The tracer is stable for up to 4 months when stored at -20°C. The standard used for the assay, adenosine 3':5'-cyclic monophosphate, may be purchased from Sigma. Samples (1-10 82 l of HCl extracts) or standards (0.04-100fmol/tube) are diluted in 50 mM Na-acetate (pH 5.5), and acetylated with 10 µl of mixture of triethylamine and acetic anhydride (2:1 vol:vol). After acetylation, cAMP antiserum (100 µl) is added from a stock solution (1:4000) made in PBS (pH 7.4), 5 mM EDTA and 1% normal rabbit serum. The tracer is diluted in PBS (pH 7.4) with 0.1% BSA, and added (20,000 cpm/tube). The assay is incubated at 4°C. overnight. The bound tracer is precipitated by adding 100 µl of goat anti-rabbit antiserum (1:20 in PBS) and 1 ml of 7% polyethyleneglycol (MW 5000-6000), centrifuging at 2000 rpm for 30 min. at 4°C. The supernatant is removed and the bound radioactivity is counted in a gamma-counter (Micromedic). To compute the cAMP data, logit calculations are performed in Excel spreadsheets. Typically, the assay sensitivity is 0.1 fmol/tube, and the standard concentration that displaces 50% of tracer is 5 fmol/tube.